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## GENETIC DIFFERENTIATION AND BIOCHEMICAL POLYMORPHISM AMONG TRICHOMONADS

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**ABSTRACT:** Isoenzyme electrophoresis was used to study levels of genetic differentiation among strains and clones of *Trichomonas gallinae*, *Trichomonas vaginalis*, *Tritrichomonas foetus*, *Tetratrichomonas gallinarum*, and *Pentatrichomonas hominis*. Strain variation was found within *T. gallinae*, *T. vaginalis*, and *T. foetus*, however, levels of enzyme polymorphism were greater in *T. gallinae* than in *T. vaginalis* or *T. foetus*. Isoenzyme genotypes were not a stable property of *T. gallinae* clones cultivated *in vitro*. Retrospective studies of *T. gallinae* SG and JB6 clones revealed that mutation occurred during *in vitro* cultivation. Heterozygotes of hexokinase-1 and phosphoglucumutase displayed 2 allomorphs in equal dosage, indicating that trichomonads are diploid for these protein loci. Phenetic clustering of the biochemical data suggests that levels of genetic divergence among the species studied are extensive.

Isoenzyme electrophoresis has proven to be a useful tool for studying protozoan genetics and systematics (Carter, 1978; Gibson et al., 1980; Kreutzer and Sousa, 1981; Chapman, 1982; Moss et al., 1986; O'Donoghue et al., 1986). Previous isoenzyme studies of trichomonads have focused on conspecific variation in isoenzyme phenotypes (Chyle et al., 1971; Takayanagi et al., 1971; Soliman et al., 1982; Coombs and North, 1983; Gradus and Matthews, 1985), although congeneric and intergeneric differences in the mobilities of certain proteins have been occasionally noted. Unfortunately, genetic interpretations of electrophoretic phenotypes and comparisons of different taxa have been lacking. In addition, investigators have not tested for correlations between isoenzyme phenotypes and virulence, geographic distribution, or antigenic properties of trichomonads.

In the present study, isoenzyme data are presented for *Trichomonas gallinae* (Rivolta), *Trichomonas vaginalis* Donné, *Tritrichomonas foetus* (Riedmüller), *Tetratrichomonas gallinarum* (Martin and Robertson), and *Pentatrichomonas hominis* (Davaine). Levels of biochemical polymorphism and genetic divergence among the above taxa are described and discussed.

### MATERIALS AND METHODS

#### Organisms

All trichomonads used in this investigation were grown from cryopreserved stabulates. The following

genera, species, strains, and clones (cl) were used (geographic origin of stabulate in parentheses): *T. gallinae* Jones' Barn (JB) strain isolate 6 (Pennsylvania, U.S.A.), JB6 cl(s) 1, 7, and 8; Stabler-gallinae (SG) cl 1 (Colorado, U.S.A.), SG scl 1 (a subclone of SG cl 1); SGC cl 1 (derived from SG cl 1 by 12 mo of continuous *in vitro* cultivation); *T. vaginalis* TvK1 strain (isolated by Kupferberg and Trussell in 1939, U.S.A.), Balt strains 23, 41, 42, 44, 53, 55, 70, and 80 (Maryland, U.S.A.), Balt 42 cl(s) 4, 5, 6, 7, 8, 9, and 11, Balt 44, cl(s) 4, 5, and 7, JH 31A strain (Maryland, U.S.A.), JH 31A, cl(s) 7, 8, and 10; *T. foetus* KV (Karlovy Vary, Czechoslovakia) cl 1 (KV-1), CB strain (Colorado, U.S.A.), UT (Utah, U.S.A.) cl 1 (UT-1), COLO cl 1 (Colorado, U.S.A.), and DK (California, U.S.A.) cl 2 (DK-2); *T. gallinarum* cl 1 (Massachusetts, U.S.A.); *P. hominis* cl 1 (Korea). Methods of isolation and histories of most of these strains and clones have been previously documented (Honigberg, 1961; Honigberg et al., 1966, 1970, 1984; Kulda, 1967; Honigberg and Goldman, 1968; Kulda and Honigberg, 1969; Stepkowski and Honigberg, 1972; Kulda et al., 1974; Su-Lin and Honigberg, 1983). The IBERG strain of *T. gallinae* was isolated on 5 May 1980 from throat swabs of a pigeon obtained from Dr. R. M. Stabler (Colorado). This strain was transferred twice in Diamond's TYM, pH 7.0, containing 1,000 U penicillin and 1,000 µg streptomycin/ml, transferred to TYM without antibiotics, and cryopreserved. Infections with the IBERG strain are usually fatal for pigeons. *Trichomonas gallinae* SG scl 1 was obtained by subcloning from SG cl 1 during the course of this investigation. An attenuated strain of *T. gallinae*, JB6 cl 8 att (attenuation confirmed by pigeon inoculation), was obtained by maintaining virulent JB6 cl 8 in culture for 144 days. *Tritrichomonas foetus* isolates CB strain and COLO cl 1 were both isolated from bulls in Colorado during 1967 and maintained as frozen stabulates.

#### Cultivation

All *T. vaginalis* strains and clones were grown in Diamond's trypticase-yeast-maltose medium (TYM) (Diamond, 1957), pH 6.0, supplemented with 10% v/v heat-inactivated horse serum. Stabulates were first cultivated in TYM and were subsequently transferred to TYM without agar. *Trichomonas gallinae*, *T. foetus*,

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TABLE I. Enzymes resolved in the trichomonad taxa.

| Abbreviation | Enzyme                            | E.C. No.  |
|--------------|-----------------------------------|-----------|
| ACP          | Acid phosphatase                  | 3.1.3.2   |
| ALD          | Fructose-biphosphate aldolase     | 4.1.2.13  |
| G6PD         | Glucose-6-phosphate dehydrogenase | 1.1.1.49  |
| HI           | Phosphohexose isomerase           | 5.3.1.9   |
| HK-1         | Hexokinase-1                      | 2.7.1.1   |
| HK-2         | Hexokinase-2                      | 2.7.1.1   |
| LDH          | Lactate dehydrogenase             | 1.1.1.27  |
| PEP-B        | Leucyl-glycyl-glycine peptidase   | 3.4.11.13 |
| MDH-1        | Malate dehydrogenase-1            | 1.1.1.37  |
| MDH-2        | Malate dehydrogenase-2            | 1.1.1.37  |
| PGM          | Phosphoglucomutase                | 5.4.2.2   |
| UAE          | Umbelliferyl acetate esterase     | 3.1.1.1   |

*T. gallinarum*, and *P. hominis* were grown in cysteine-tryptose-liver-maltose medium (CTLM) (McEntegart, 1952), pH 7.3, or Diamond's TYM, pH 7.0 (modified by substituting BBL trypticase peptone for trypticase), media without agar, supplemented with 10% heat-inactivated horse serum. Log-phase trichomonads were harvested by centrifugation and subsequently washed 3 times in 10 volumes of an ice-cold homogenizing solution containing 6 g sucrose and 10 mg each of dithiothreitol,  $\beta$ -NAD, and  $\beta$ -NADP/100 ml of distilled water. Washed organisms were pelleted at 11,600 g and frozen at  $-70^{\circ}\text{C}$ . Frozen samples were used within 3 mo of the time of harvest.

#### Electrophoresis

Immediately before use, cell pellets were thawed, suspended in 2 volumes of homogenizing solution, and disrupted using either a probe sonicator or a Dounce homogenizer. Homogenates were centrifuged at 11,600 g for 3 min to remove cell debris, and samples of the supernatant fluid were subjected to vertical starch gel electrophoresis at  $4^{\circ}\text{C}$ . The following buffers were used for electrophoresis: (a) phosphate-citrate, pH 6.8 (60 mM  $\text{Na}_2\text{HPO}_4$ , 10 mM citric acid) diluted 1:4 for gel and undiluted for electrode bath; (b) Tris-maleate, pH 7.5 (100 mM Tris adjusted to pH 7.5 with 2 M maleic acid) diluted 1:3 for gel and undiluted for electrode bath.

#### Enzyme localization

Following electrophoresis, proteins were visualized in gel slices by procedures described in Harris and Hopkinson (1976). Acid phosphatase was detected by the method of El-Sharkawy and Huisinsh (1971). See Table I for enzymes resolved, their abbreviations, and Enzyme Commission numbers. Control gel slices developed without the corresponding specific substrates (e.g., lactate dehydrogenase without sodium lactate), yielded no detectable stain reactions.

Differences in phenotypes at a specific locus were used to deduce genotypes of clones or the number of allomorphs occurring in noncloned strains. For each locus, the allomorph of fastest anodal mobility was designated A; then, the others in order of decreasing mobilities, as B, C, etc. When more than 1 presumed locus yielded products with the same enzymatic activity, the locus with isozymes of faster anodal mobility was designated 1 and that of slower mobility as 2.

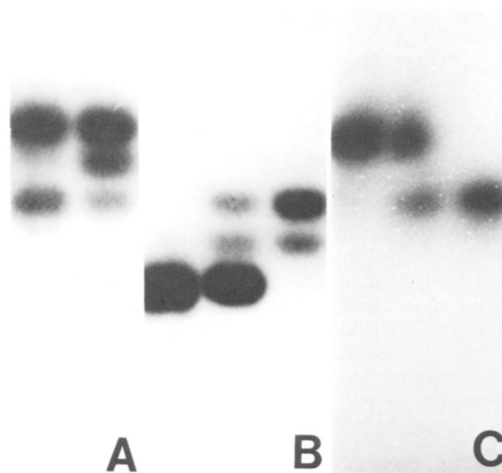


FIGURE 1. Phenotypes of some polymorphic trichomonad enzymes. The anode is at the top. Taxa and their presumed genotypes (in italics) are read left to right. Methods of allomorph designation are described in the Materials and Methods. A. Hexokinase loci (HK) 1 and 2. *Trichomonas gallinae* JB6 cl 7, HK-1 CC, HK-2 CC; *T. gallinae* JB6 cl 1, 1985 stabilate, HK-1 CD, HK-2 CC. The HK-1 heterozygote has 2 bands with a 1:1 staining intensity, indicating a monomeric subunit structure. B. Phosphoglucomutase (PGM). *T. gallinae* JB6 cl 7, PGM DD; *T. gallinae* JB6 cl 1, 1985 stabilate, PGM BCD; *T. gallinae* SGC cl 1, PGM BC. Heterozygote phenotype characteristic of a monomeric protein. C. Leucyl-glycyl-glycine peptidase (PEP-B). *Pentatrichomonas hominis* cl 1, PEP-B AA; *Trichomonas vaginalis* Balt 80, PEP-B AB; *T. gallinae* IBERG, PEP-B BB.

#### Data analysis

The BIOSYS-1 computer program (Swofford and Selander, 1981) was used to analyze genetic data and produce the UPGMA phenogram. Clustering procedures were performed using only those 9 loci that were scored in all species (i.e., excluding the proteins acid phosphatase, lactate dehydrogenase, and umbelliferyl acetate esterase in Table II).

#### RESULTS

Representative isoenzyme phenotypes and their corresponding genotypes are shown in Figure 1. Additional enzymes that showed activity on gel slices, but were not adequately resolved for interpretation included: glutamate-oxaloacetate transaminase, glutamate dehydrogenase,  $\alpha$ -naphthyl acetate esterase, superoxide dismutase, and catalase. In a few instances, a protein resolved in one species or strain lacked sufficient activity to be interpreted in another. In other cases, a protein with more than 1 locus in one species sometimes showed only a single activity zone in another. In such instances, the single

zone of activity was considered to be composed of 2 comigrating loci.

Malate dehydrogenase-1 and -2 were the only loci with more than 1 phenotype in *T. foetus*, and they served to distinguish KV-1 from the other strains and clones. Polymorphic loci in *T. gallinae* included: hexokinase-1, hexokinase-2, phosphoglucosmutase, malate dehydrogenase-1, and malate dehydrogenase-2. Heterozygotes were observed for *T. gallinae* hexokinase-1 and phosphoglucosmutase only (Fig. 1A, B). Polymorphic loci in *T. vaginalis* were restricted to phosphoglucosmutase and leucyl-glycyl-glycine peptidase (Fig. 1C) in Balt 41 and Balt 80 strains, respectively. This was the only protein variation detected among the 23 stabilates of this species examined in the present study. These stabilates represented 9 strains, 13 clones obtained from these strains, and 1 strain (TvK1) that was isolated in 1939.

Studies of extant clones of *T. gallinae* JB6 indicate that they are not genetically identical (Table II). For example, JB6 cl 1 has a CD genotype for hexokinase-1, whereas JB6 clones 7 and 8 have a CC genotype at this locus. The parent strain of these clones, JB6, also has a CC hexokinase-1 genotype and is identical to clones 7 and 8 at all other loci. This suggests that either the D allomorph was lost from the parent strain subsequent to cloning, or that a new allomorph is being expressed in JB6 cl 1. In support of the second explanation, retrospective studies revealed that isolates of JB6 cl 1 and SG cl 1 have undergone genetic changes during *in vitro* cultivation. For example, a 1970 stabilate of JB6 cl 1 yielded a homozygous DD phosphoglucosmutase genotype, however, a 1985 stabilate of the same clone yielded a BCD phosphoglucosmutase phenotype with a complex gene dosage (Fig. 1B). In the case of SG cl 1, stabilates of the original clone yielded a BCD phosphoglucosmutase phenotype, indicating that this was not a successful clone, but instead a mixed population. This was substantiated by subcloning, which yielded SG scl 1 with a DD phosphoglucosmutase genotype. In contrast to the situation with JB6 cl 1, prolonged cultivation of SG cl 1 (i.e., SGC cl 1) produced a normal dosage phosphoglucosmutase BC genotype (Fig. 1B).

The number and relative staining intensity of isozyme bands in clones heterozygous for individual protein loci were used to deduce the subunit structures of those enzymes. Heterozygotes of hexokinase-1 and phosphoglucosmutase dis-

played 2 bands of equal staining intensity, indicating that these proteins are monomers (Fig. 1A, B).

Some trichomonad strains can be conclusively diagnosed from their isoenzyme phenotypes (Table II). Among *T. gallinae* strains, the hexokinase-2 locus "C" allele distinguishes the virulent JB and IBERG strains from the avirulent SG strain. In addition, the highly virulent *T. foetus* KV-1 isolate can be distinguished from those clones and strains of intermediate and low virulence (DK-2, CB, UT-1, and COLO cl 1) by the malate dehydrogenase-1 and malate dehydrogenase-2 loci. In contrast, no differentiating allomorphs were detected among *T. vaginalis* Balt strains that differed in their inherent virulence as previously evaluated on the basis of the pathologic changes they caused in women, and by the subcutaneous mouse assay (Honigberg et al., 1984). Finally, a stabilate of *T. gallinae* JB6 cl 8, which was virulent for pigeons, had an isoenzyme profile identical to a stabilate of JB6 cl 8 that had been attenuated by 144 days of *in vitro* cultivation.

A matrix of Rogers' (1972) genetic similarity and Nei's (1972) genetic distance coefficients among representative taxa are presented in Table III. Pairwise comparisons of species yielded extremely low genetic similarity levels. Phenetic clustering (UPGMA) of the genetic data (Fig. 2) produced 3 major groups. *Pentatrachomonas hominis* showed the lowest level of genetic similarity to the other trichomonad taxa. The 2 urogenital species of mammals (*Trichomonas vaginalis* and *Tritrichomonas foetus*) and the 2 gastrointestinal species from birds (*Trichomonas gallinae* and *Tetratrachomonas gallinarum*) clustered as 2 distinct groups. Within *T. gallinae*, the 2 isolates from Colorado (IBERG and SG cl 1) showed low levels of genetic similarity in comparison to the isolate from Pennsylvania (JB6).

## DISCUSSION

Differences in levels of enzyme polymorphism were found between *Trichomonas gallinae* and *Trichomonas vaginalis*. Five of 11 loci surveyed were polymorphic in *T. gallinae*, but only 2 of 11 loci were polymorphic in *T. vaginalis*. Two polymorphic loci were detected in *Tritrichomonas foetus*, however, only 5 stabilates representing 5 strains were surveyed. Alderete (1983) also reported homogeneity in the protein profiles of 5 strains of *T. vaginalis* analyzed by SDS-PAGE. The level of *T. vaginalis* enzyme poly-

TABLE II. *Electrophoretic genotypes in trichomonad taxa.\**

| <i>Trichomonas gallinae</i> |     |                                  |                    |                    |       |         |          |          |
|-----------------------------|-----|----------------------------------|--------------------|--------------------|-------|---------|----------|----------|
| Locus                       | JB6 | JB6 cl(s) 7, 8<br>JB6 cl 8 (att) | JB6 cl 1<br>(1970) | JB6 cl 1<br>(1985) | IBERG | SG cl 1 | SG scl 1 | SGC cl 1 |
| ACP                         | n/a | n/a                              | n/a                | n/a                | n/a   | n/a     | n/a      | n/a      |
| ALD                         | DD  | DD                               | DD                 | DD                 | DD    | DD      | DD       | DD       |
| G6PD                        | BB  | BB                               | BB                 | BB                 | BB    | BB      | BB       | BB       |
| HI                          | CC  | CC                               | CC                 | CC                 | CC    | CC      | CC       | CC       |
| HK-1                        | CC  | CC                               | CD                 | CD                 | CC    | CD      | CC       | DD       |
| HK-2                        | CC  | CC                               | CC                 | CC                 | CC    | DD      | DD       | DD       |
| PEP-B                       | BB  | BB                               | BB                 | BB                 | BB    | BB      | BB       | n/a      |
| MDH-1                       | BB  | BB                               | BB                 | BB                 | AA    | AA      | AA       | AA       |
| MDH-2                       | DD  | DD                               | DD                 | DD                 | BB    | BB      | BB       | BB       |
| PGM                         | DD  | DD                               | DD                 | BCD                | DD    | BCD     | DD       | BC       |
| UAE                         | n/a | BB                               | n/a                | n/a                | n/a   | n/a     | n/a      | n/a      |
| LDH                         | AA  | AA                               | AA                 | AA                 | AA    | AA      | AA       | n/a      |

\* Locus abbreviations are indicated in Table I; n/a = no activity detected.

morphism observed in the present study conflicts with the results of Soliman et al. (1982), who reported variation in 4 enzymes (lactate dehydrogenase, malate dehydrogenase, hexokinase, and phosphohexose isomerase) among clones derived from 32 strains of *T. vaginalis*. These 4 loci were monomorphic in the strains of *T. vaginalis* we studied. Limited variability at the phosphoglucumutase locus was detected by Soliman et al. (1982) and in the present investigation. Clearly, enzyme polymorphism in the *T. vaginalis* isolates we examined was considerably lower than that found by Soliman et al. (1982) or by us for *T. gallinae*. At this time, explanations for such differences remain speculative; however, some possibilities merit discussion. First, the isolates we studied may not be representative of the genetic variability present in *T. vaginalis*. All of the strains and clones we surveyed, except for TvK1, were obtained from patients attending the Johns Hopkins Hospital Gynecologic or Obstetric Clinics in Baltimore, Maryland, U.S.A. A comparison of strains isolated from women residing in distant geographic localities may reveal additional variation. Second, very little is known about what effects *in vitro* cultivation has on the genetic composition of isolates. Culture medium could act as a highly selective environment, or conversely, an unrestrictive one, which supports the growth of mutants that would not normally survive *in vivo*. One possibility is that *T. gallinae*, which usually grows more vigorously than *T. vaginalis in vitro*, may experience less selection pressure or fewer population bottlenecks than *T. vaginalis* upon initial isolation. Thus, variability

of *in vivo* strains of *T. gallinae* may be better preserved upon *in vitro* isolation. It should also be noted that differences at the hexokinase-1 locus between JB6 clone 1 (1970) and clones 7 and 8 may reflect allomorphic variation that was once present in the parent strain from which these clones were derived. Isoenzyme variation among clones of *Trypanosoma cruzi* derived from a single parental stock has been reported (Goldberg and Silva Pereira, 1983).

This investigation also demonstrates that isoenzyme genotypes are not stable attributes of *in vitro*-cultivated *T. gallinae*. From the observations with *T. gallinae* JB6 clones, it appears that *in vitro* mutation influences the extant isoenzyme phenotypes of clones. For example, following an undefined period of *in vitro* cultivation, JB6 cl 1 began expressing a phosphoglucumutase allomorph that was not detected in cultures prepared from the 1970 stabilate of that clone. This allomorph, which was also found in other clones of JB6, probably originated by mutation. Changes in isoenzyme patterns and virulence during axenization of *Entamoeba histolytica* have also been reported (Mirelman et al., 1986).

Changes in the composition of *T. gallinae* populations were observed with SG "clone 1." Isoenzyme study revealed that cultures inoculated with the original SG cl 1 stabilate had a complex BCD phosphoglucumutase phenotype, which implies that it was not a clone, but rather a mixed population. This was substantiated by the subclone SG scl 1 that had a homozygous DD phosphoglucumutase genotype. Only by additional subcloning from SG cl 1 could it be ascertained

TABLE II. *Extended.*

| <i>Trichomonas vaginalis</i> |   | <i>Tritrichomonas foetus</i> |      |                                 |                                       |  |
|------------------------------|---|------------------------------|------|---------------------------------|---------------------------------------|--|
| Balt 41                      | Balt 42 cl(s) 4, 6, 7, 8, 9, 11<br>Balt 23, 42, 44, 53, 55, 70<br>Balt 44 cl(s) 4, 5, 7<br>JH31-4<br>JH31-4 cl(s) 1, 3, 5<br>TvKl | Balt 80                      | KV-1 | COLO cl 1<br>CB<br>DK-2<br>UT-1 | <i>Pentatricho-<br/>monas hominis</i> | <i>Tetratricho-<br/>monas gallinarum</i> |
|                              |   |                              |      |                                 | cl 1                                  | cl 1                                     |
| BB                           | BB  | n/a                          | n/a  | n/a                             | AA                                    | AA                                       |
| DD                           | DD  | DD                           | AA   | AA                              | CC                                    | BB                                       |
| AA                           | AA  | AA                           | CC   | n/a                             | CC                                    | CC                                       |
| BB                           | BB  | BB                           | DD   | DD                              | AA                                    | CC                                       |
| EE                           | EE  | EE                           | AA   | AA                              | BB                                    | DD                                       |
| EE                           | EE  | EE                           | AA   | AA                              | BB                                    | FF                                       |
| BB                           | BB  | AB                           | BB   | n/a                             | AA                                    | BB                                       |
| BB                           | BB  | BB                           | BB   | CC                              | DD                                    | AA                                       |
| CC                           | CC  | CC                           | CC   | EE                              | FF                                    | AA                                       |
| AB                           | BB  | BB                           | AA   | AA                              | AA                                    | DD                                       |
| n/a                          | n/a   | n/a                          | AA   | n/a                             | n/a                                   | n/a                                      |
| CC                           | CC  | CC                           | n/a  | n/a                             | BB                                    | CC                                       |

how many other genotypically distinct populations are present within this culture. Interestingly, during 12 mo of *in vitro* cultivation, SG cl 1 phosphoglucosmutase and hexokinase-1 phenotypes changed. As can be seen from the SGC cl 1 data (Table II), the SG cl 1 mixed population became monomorphic for hexokinase-1 and "heterozygous" for phosphoglucosmutase. In this case, no new allomorphs were expressed, and it is unknown whether a subpopulation containing the other allomorphs remains as a hidden component of the SGC cl 1 population.

Within *Trichomonas gallinae* and *Tritrichomonas foetus*, some strains could be definitively diagnosed on the basis of their isoenzyme phenotypes. For example, *T. gallinae* SG, IBERG, and JB6 strains can be differentiated from one another. In addition, the strains and clones of *T. gallinae* that were isolated from virulent stocks differed in their hexokinase-2 genotype from the avirulent SG clones. *Tritrichomonas foetus* KV1 has malate dehydrogenase mobility differences

that differentiate it from all other strains of this species examined. These malate dehydrogenase allomorphs differentiate the highly virulent *T. foetus* KV-1 isolate from moderately virulent and avirulent strains and clones. However, for both *T. gallinae* and *T. foetus*, a limited number of strains with known virulence levels have been examined, and therefore it is premature to conclude that these genetic markers are correlated with virulence. Unfortunately, isoenzymes did not differentiate among *T. vaginalis* strains that varied considerably in their inherent pathogenicity (Honigberg et al., 1984).

The UPGMA phenogram (Fig. 2) shows the relative degree of genetic differentiation among the taxa examined by us, and should not be interpreted as representing phylogenetic relationships. Rogers' (1972) genetic similarity coefficients among these taxa are very low, which suggests that times of divergence among these taxa are ancient. Phenetic clustering of the genetic data results in associations that are discor-

TABLE III. *Matrices of Rogers' similarity values (above the diagonal) and Nei's distance coefficients (below the diagonal) for representative trichomonad taxa.*

| Taxon                                       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 <i>Trichomonas gallinae</i> JB6           | —     | 0.944 | 0.778 | 0.667 | 0.348 | 0.333 | 0.278 | 0.333 | 0.0   | 0.222 |
| 2 <i>Trichomonas gallinae</i> JB6 cl 1 1970 | 0.029 | —     | 0.722 | 0.611 | 0.363 | 0.348 | 0.293 | 0.389 | 0.015 | 0.237 |
| 3 <i>Trichomonas gallinae</i> IBERG         | 0.251 | 0.297 | —     | 0.889 | 0.237 | 0.222 | 0.167 | 0.444 | 0.0   | 0.111 |
| 4 <i>Trichomonas gallinae</i> SGcl 1        | 0.405 | 0.464 | 0.118 | —     | 0.237 | 0.222 | 0.167 | 0.444 | 0.0   | 0.111 |
| 5 <i>Trichomonas vaginalis</i> BALT 41      | 1.070 | 1.041 | 1.475 | 1.475 | —     | 0.944 | 0.889 | 0.126 | 0.056 | 0.389 |
| 6 <i>Trichomonas vaginalis</i> BALT 42      | 1.099 | 1.070 | 1.504 | 1.504 | 0.029 | —     | 0.944 | 0.111 | 0.0   | 0.333 |
| 7 <i>Trichomonas vaginalis</i> BALT 80      | 1.252 | 1.224 | 1.763 | 1.763 | 0.061 | 0.029 | —     | 0.056 | 0.056 | 0.278 |
| 8 <i>Trichomonas gallinarum</i> cl 1        | 1.099 | 0.916 | 0.811 | 0.811 | 2.169 | 2.197 | 2.862 | —     | 0.111 | 0.222 |
| 9 <i>Pentatrichomonas hominis</i> cl 1      |       |       |       |       | 2.862 |       | 2.862 | 2.197 | —     | 0.222 |
| 10 <i>Tritrichomonas foetus</i> KV 1        | 1.504 | 1.475 | 2.197 | 2.197 | 0.916 | 1.099 | 1.252 | 1.504 | 1.504 | —     |

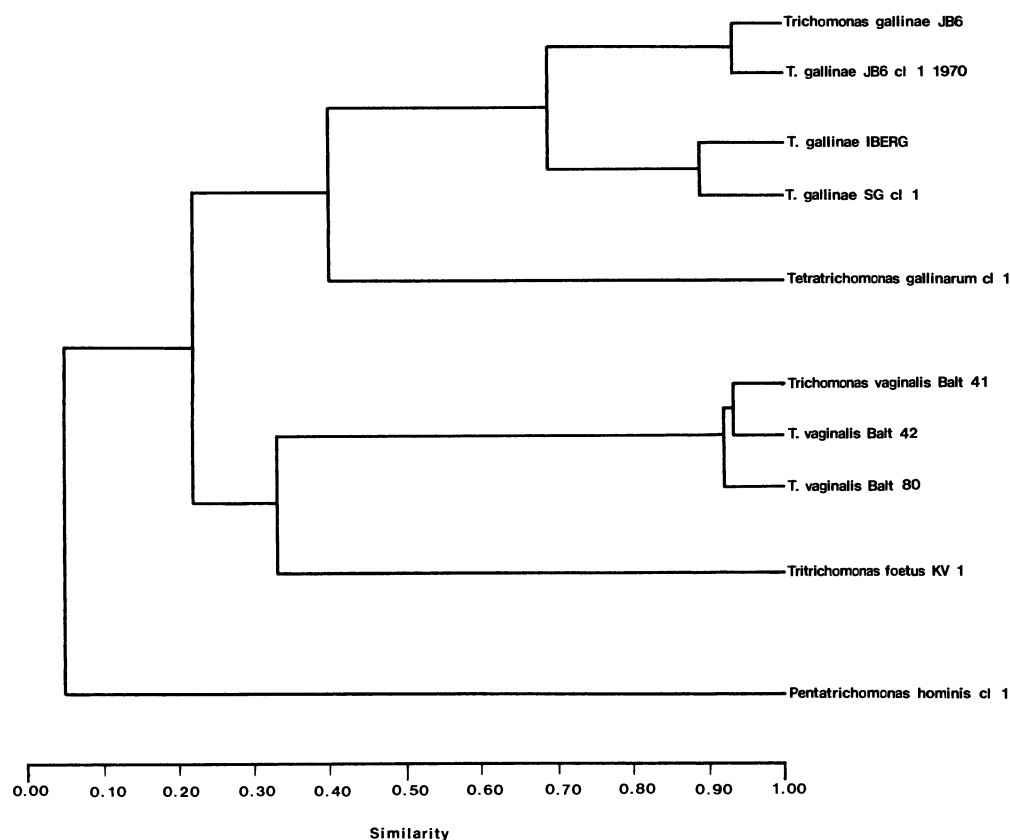


FIGURE 2. Phenogram of genetic differentiation among representative trichomonad taxa. Rogers' similarity metric was clustered by the UPGMA method. The cophenetic correlation coefficient for the phenogram is 0.966.

dant with both alpha-taxonomy and limited immunological data (Honigberg, 1978; Stepkowski and Honigberg, 1981). For example, the congeners *T. gallinae* and *T. vaginalis* do not cluster together in the phenogram, but instead are grouped with *Tetratrichomonas gallinarum* and *Tritrichomonas foetus*, respectively. It is noteworthy that phenetic clustering of the genetic data places the 2 urogenital species from mammals and the 2 species from the digestive tract of birds into 2 distinct groups. However, the low levels of genetic similarity among the species studied indicate that studies of more conservative molecules may be required to clarify the phylogenetic relationships in this group. Finally, the low levels of genetic similarity between *T. gallinae* strains isolated in Colorado and Pennsylvania suggest that there may be significant geographic variation in this species.

Subunit structures of the proteins hexokinase-1 and phosphoglucomutase in *T. gallinae* correspond to those of a phylogenetically diverse array

of animals (Dessauer and Braun, 1982), as do those of other protozoa (Chapman et al., 1984). The expression of 2 allomorphs in equal dosage as observed in heterozygous clones suggests that trichomonads are diploid for these protein loci. However, this does not necessarily imply that they are chromosomally diploid. Studies of a similar nature have led investigators to conclude that *Trypanosoma cruzi* (Tibayrenc et al., 1981; Miles, 1983) and *Trypanosoma brucei* (Gibson et al., 1985; Jenni et al., 1986) are also diploid at many loci coding for enzymatic proteins.

Difficulties in resolving certain loci in some trichomonad taxa (see also Soliman et al., 1982) have many potential causes. No lactate dehydrogenase activity was found in *T. foetus* strains, however a recent report (Steinbuechel and Muller, 1986) indicates that this species does not produce lactate as a metabolic end product. Some enzymatic activities, hexokinase-2 in *T. vaginalis* for example, were lost relatively rapidly following homogenization. Presumably this locus and cer-

tain others were either thermolabile or susceptible to proteinases released on homogenization (Coombs and North, 1983). Activation of trichomonad proteases by dithiothreitol in the homogenizing fluid may also have contributed to the observed reduction of some enzymatic activities.

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